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Effect of Various Alcohols on the Intramuscular Absorption of Isonicotinamide in the Rat[1]

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The effect of various alcohols on the intramuscular absorption of water-soluble drug was studied using isonicotinamide as a model compound. It was found that linearity of the disappearance curves of the drug was maintained in the presence of the tested alcohols. The absorption inhibitory effect was also demonstrated when other alcohols of different classes were used, and it was increased with the increase of the molecular weight of the monohydric alcohols. Good correlations were demonstrated not only between the absorption inhibitory effect and denaturating power but also between the absorption inhibitory effect and the hemolytic effect of alcohols.

Keywords——intramuscular absorption; rat thigh muscle; isonicotinamide; effect of monohydric alcohols; absorption inhibitory effect

In a previous paper[1b] we have shown that the intramuscular absorption of water-soluble drugs was remarkably inhibited in the presence of ethanol. The reduction in the absorption of these drugs was reflected on their plasma concentrations, and the inhibition caused by the presence of ethanol was found to be concentration dependent. The inhibitory effect of ethanol was ascribed to an inhibition in drug permeation through extracellular spaces and connective tissues.

Since alcohols other than ethanol such as benzyl alcohol and isopropanol are widely used as pharmaceutical solvents in parenteral preparation, the present study was carried out to investigate the effect of various alcohols on the intramuscular absorption of drugs.

Experimental

Materials——Isonicotinamide was of analytical grade and obtained commercially. Alcohols studied were methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, iso-butanol, *tert*-butanol and benzyl alcohol.

Preparation of Injectable Solutions——Solutions of isonicotinamide (50 mM) for intramuscular administration was prepared in physiologic saline solution and adjusted by 1 N HCl or 1 N NaOH at pH 7.0.

Absorption Experiments——Male Wistar albino rats weighing 180—220 g were used. Preparation of animal and injection technique were mentioned previously.[3]

Analytical Methods——Isonicotinamide was determined spectrophotometrically as described previously.[3]

Results and Discussion

The effect of alcohols and dose-response relation on the absorption of isonicotinamide was studied. Figure 1 shows the disappearance of isonicotinamide from the site of injection after its intramuscular administration in the presence of 0.5 and 1.0% (v/v) benzyl alcohol or 20.0% (v/v) isopropanol. As is evident from the figure the disappearance curves are linear and their linearity was maintained irrespective of the presence of alcohols. Other alcohols

1) *a*) This paper constitutes the 15th report in a series of "Biopharmaceutical Studies on Parenteral Preparations"; *b*) Preceding paper, Part XIV: H. Kobayashi, Y. Miyoshi, K. Kitamura, Y. Yoshizaki, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **25**, 2862 (1977).

2) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*

3) H. Kobayashi, T. Nishimura, K. Okumura, S. Muranishi, and H. Sezaki, *J. Pharm. Sci.*, **63**, 580 (1974).

were also tested and gave a similar results, data not shown here, which demonstrate that the disappearance of isonicotinamide from the site of injection in the presence of other alcohols is apparently described by a pseudo first-order process as was found with ethanol.[1b] Also, it shows that the absorption of isonicotinamide was reduced when benzyl alcohol or isopropanol was included in the formulation. Moreover a more inhibition can be noticed on increasing the concentration of benzyl alcohol.

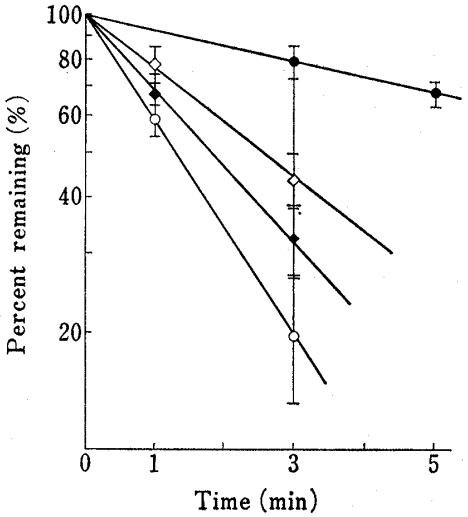

Fig. 1. Effect of Alcohols on the Disappearance of Isonicotinamide from the Rat Thigh Muscle

Each point represents the mean value of at least five animals. Vertical bars indicate standard deviation, and straight lines are the result of least-square regression analysis.

Key: ○, control (saline); ◆, 0.5% (v/v) benzyl alcohol; ◇, 1.0% (v/v) benzyl alcohol; ●, 20.0% (v/v) isopropyl alcohol.

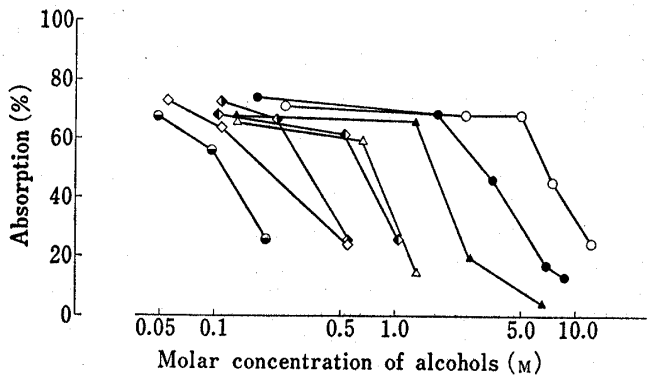

Fig. 2. Effect of Alcohols on the Absorption of Isonicotinamide from the Rat Thigh Muscle

Each point represents the mean value of at least five animals. A 10 μl of 50 mM isonicotinamide in various concentrations of alcohols was injected intramuscularly and the amount absorbed was determined after 3 minutes.

Key: ○, methanol; ●, ethanol; △, *n*-propanol; ▲, isopropanol; ◇, *n*-butanol; ◐, iso-butanol; ◑ *tert*-butanol; and ◒ benzyl alcohol.

Then the effect of various alcohols was investigated. As is obvious in Fig. 2, the absorption inhibitory effect of alcohols is not observed untill their concentration is increased to a certain level which is varied according to the alcohol used, at that level a remarkable inhibition on the intramuscular absorption of isonicotinamide occurred. It has also shown that the absorption inhibitory effect is not specific to ethanol. Consequently the mechanism of the absorption inhibitory effect of other alcohols can be expected to be eventually similar to that of ethanol.

As far as the alcohols used in this study are concerned, it is evident that their absorption inhibitory effect is influenced by the number of carbon atoms they have. In the case of primary alcohols the more the number of carbon atoms, the more the inhibition on the absorption of isonicotinamide occurred. Moreover, among alcohols which have the same number of carbon atoms secondary alcohols showed a less inhibitory effect and tertiary alcohols even lesser than that of the primary alcohols. This can be seen by comparing the inhibitory effect of *n*-butanol, isobutanol and *tert*-butanol which represent primary, secondary and tertiary alcohols respectively and possessing the same number of carbon atoms.

Herskovits, *et al.*[4] studied the solvent denaturation of myoglobin and demonstrated that judging from the midpoints of the denaturation trasition of water-miscible alcohols, ureas and amides, the effectiveness of these denaturating agents on sperm-whale myoglobin increases with increasing chain length and hydrocarbon content. Furthermore, Ku, *et al.*[5]

4) T.T. Herskovits and H. Jaillet, *Science*, **163**, 282 (1969).
5) S. Ku and D.E. Cadwallader, *J. Pharm. Sci.*, **63**, 60 (1974).

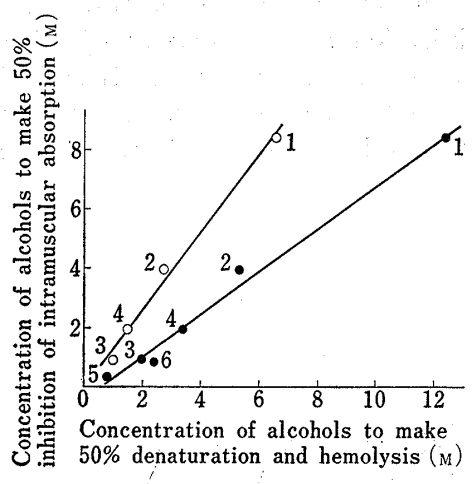

Fig. 3. Relationship between the Intramuscular Absorption Inhibitory Effect and other Biological Effects of Alcohols

The molar concentration of alcohols to make 50 % inhibition in the intramuscular absorption of isonicotinamide was calculated from the data of Fig. 2, and that to make 50% denaturation or hemolysis was calculated from a published data of Herskovits, *et al.*[4] and Ku, *et al.*,[5] Cadwallader, *et al.*[6] respectively.

Straight lines are the result of least-square regression analysis.

Key: ●, intramuscular absorption and denaturation ($y=0.75x-0.490$, $r=0.994$); ○, intramuscular absorption and hemolysis ($y=1.333x-0.939$, $r=0.995$). 1, methanol; 2, ethanol; 3, *n*-propanol; 4, isopropanol; 5, *n*-butanol: and 6, *tert*-butanol.

reported that from the results of the behavior of erythrocytes in various water-monohydric alcohol solutions, hemolytic activity increases with an increase in molecular weight of monohydric alcohols, and the isomeric alcohols were noted to possess weaker hemolytic activity then the corresponding normal alcohol.

Therefore, we have studied the relationship between the intramuscular absorption inhibitory effect and other biological effects of alcohols. The molar concentration of various alcohols required to produce a 50% inhibition in the intramuscular absorption of isonicotinamide was determined, and its relation to that producing a 50% in either myoglobin denaturation or hemolysis of human red blood cells (calculated from a previously published data of Herskovits, *et al.*[4] or Ku, *et al.*[5] and Cadwallader, *et al.*[6] respectively) was investigated.

As is shown in Fig. 3, the absorption inhibitory effect of various alcohols has a good correlation with their denaturating power and hemolytic effect. These results suggest that the inhibition of the diffusion of drug molecules through the connective tissues is partly affected by protein denaturation. It can be concluded that not only ethanol but also other alcohols of different classes inhibit the intramuscular absorption of isonicotinamide.

6) D.E. Cadwallader, B.W. Wickliffe, and B.L. Smith, *J. Pharm. Sci.*, **53**, 927 (1964).